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Association Studies between Dental Caries among Smokers and Cytogenetic Changes

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Abstract

The many constituents in cigarette smoke induce chromosomal abnormalities, gene mutations, DNA strand breaks, and micronuclei, all of which lead to the carcinogenic impacts caused by cigarette smoke. The objective of this study was to investigate the relationship between DMFT and cytogenetic alterations (cytotoxicity and mutagenicity) in buccal mucosal cells caused by cigarette smoke in young male using a micronucleus test and the Giemsa staining method. 70 men between the ages of 30 and 35 participated in this study. Thirty-five (smokers) and thirty-five (non-smokers). Giemsa stain was utilized after the smears were collected using a cytobrush. DMFT was used to determine dental caries. Smokers and nonsmokers have significantly different micronuclei along with other abnormalities ($P<0.05$). The DMFT mean is not statistically significant ($P>0.05$). Dental caries (DMFT) and micronucleus expression are not significantly correlated with one exception of a weakly significant association between PK and DMFT. Cytogenetic evaluation of buccal cells by micronucleus assay is a good indicator to identify people who are highly susceptible to oral mutations due to the detrimental impacts of smoking. The genotoxic effects of smoking lead to increased expression of micronuclei and a lack of oral health.

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Introduction

Buccal cells act as the initial barrier for inhalation or ingestion pathways and can metabolize carcinogens into reactive products. Since it is estimated that over 90% of human cancers are of epithelial origin, it can be believed that oral epithelial cells serve as the main target for early genotoxic events triggered by carcinogenic agents entering the body through inhalation and ingestion [1].

A significant amount of research has been directed towards the exploration of new non-invasive techniques that facilitate the prediction of the timing and extent of malignant transformation in oral mucosa cells due to environmental mutations [2].

The micronucleus assay is a good, minimal invasive procedure to examine DNA and chromosomal damage, cytokinetic defects, the tissue's reparative capacity, and cell mortality in exfoliated buccal cells [3].

The test's specificity for identifying genotoxic and cytotoxic effects is enhanced by examining additional degenerative nuclear changes that signify cell death [4].

The micronucleus test scoring can serve as a valuable biomarker for the early diagnosis and screening of the genotoxic effects of cigarette smoking, as well as a primary preventive method for different smoking-induced diseases [5].

Smoking is a public health issue that negatively affects oral and overall health, resulting in higher rates of illness and death [6]. WHO estimates that tobacco use kills over six million people around the world each year, and many of these mortality rates happen prematurely [7].

Cigarette smoke contains a lot of different chemicals that might cause cancer by breaking DNA strands, adding oxygen to DNA, changing genes, causing chromosomal aberrations, and forming micronuclei in different systems [8].

The process of caries is dynamic process, with periods of demineralization and remineralization of the tooth structure that are linked to changes in the pH of the plaque biofilm.

In general, the lower the pH, the more likely it is that the hard tissue parts will dissolve. If the

pH in the biofilm stays below a certain level for a long time after eating free sugars, the tooth's mineral part will slowly lose calcium and phosphate, which will lead to gradual demineralization. In the initial (subclinical) phases and even when sufficient mineral loss has resulted in the clinical manifestation of a white spot on the tooth surface [9].

The link between smoking and a higher risk of caries is attributed to a decrease in salivary function, which helps protect teeth. Furthermore, exists a difference in salivary buffering capacity between smokers and nonsmokers, which correlates with the risk of caries [10]. On the other hand, a study indicated that smoking tobacco could lower the possibility of cavities because thiocyanate, a chemical in tobacco, helps prevent cavities [11].

The modification of eating habit of the smoker tend to have high amount of sugar containing soft drink and snacks, also tobacco smokers prefer high consumption of dietary sugar leading to increase the cariogenic activity, furthermore, heavy smokers tend to have high amount of tea and coffee that contain sugar and also adding sweeteners during the tobacco manufacturing process (range 4–13%) could contribute to dental caries [12,13].

Materials and Methods

Seventy male volunteers between the ages of thirty and thirty-five were visiting AL- Kut specialized center for dentistry. Of these, thirty-five were heavy smokers, meaning they had smoked minimally twenty cigarettes a day for five years and never gone more than three months without smoking in the previous years, and thirty-five were non-smokers. They did not take any supplements and did not have any systemic health conditions.

The volunteers' smoking status was evaluated using a self-reported questionnaire that asked about their demographics, oral health, smoking status, and cytopathological findings. Dental caries was measured by DMFT (D = decayed, M = missed, F = filled, and T = number of teeth) for permanent teeth as part of the oral examinations, and the results were categorized in accordance with WHO (2013) [14]. Next, a smear was obtained from intact buccal mucosa using cytopathological brush. The smears were spread out on a glass slide that had been cleaned, dried, and labeled. Each slide has the patient's name and code inscribed on it. Giemsa stain was used after the slides had air dried [15]. A light microscope was used to examine the slides at a 40x magnification. A zigzag pattern of 1000 cells per slide was used to check for the presence of micronuclei and other abnormalities, including, cells with condensed chromatin (CC), pyrotechnic cells (PK), kyrolysis cells (KL), nuclear buds (NB) karyorrhectic cells (KR), and micronucleated cells (MN) [16].

The SPSS software (SPSS version 26) was used to analyze the data. Pearson's correlation coefficient test, means, standard deviation, and independent sample t-test were performed.

Results

There was no difference in DMFT between smokers and non-smokers (DMFT mean = 13.82 for smokers and 13.02 for non-smokers (p=0.5).

Smokers had significantly higher (P<0.05) means of micronucleated cells (MN), pyknotic cells (PK), and karyolysis cells (KL) than non-smokers. There were no statistically significant variations between the study and control groups in the means of nuclear Buds (NB), karyorrhectic cells (KR), and binucleated cells (BN) (Table 1). Since neither the smokers nor the nonsmokers had any cells with condensed chromatin, there was no statistically significant difference.

A weak significant negative correlation between PK and DMFT (r=-0.399, P=0.018) was identified in the non-smokers group, but no statistically significant correlations (P > 0.05) were found between DMFT and micronuclei expression in both groups (Tables 2 and 3).

Discussion

The mean of caries experience (DMFT) in the present study was calculated from the WHO oral health survey (2013) [14]. The findings indicated a higher prevalence among smokers compared to non-smokers, while the differences were not statistically significant (P>0.05), corroborating some of studies [17]. Conversely, this result conflicts with previous investigations, that identified significant variances in caries experience between smokers and non-smokers [18,19]. However, it is important to consider other factors that affect developing dental caries, such as smoking, chronological age, oral hygiene behaviors, nutritional patterns, preventive dental checks, general health requirements, and the degree of dental education. Consequently, determining the exact link between dental caries and smoking is challenging, as dental caries is a multifactorial disease [20]. People that smoke usually consomme a lot of sugary foods. While tobacco smoking elevates the risk of dental caries, an exact mechanism for this correlation has not been found. This could be due to a lack of uniformity among the studies. Consequently, comprehensive research is necessary to clarify this association [21].

Micronucleus expression variables (MN, PK, and KL) were significantly elevated in smokers compared to non-smokers, confirming multiple studies that indicate cigarette smoking and other tobacco use raise the frequency of micronuclei in isolated buccal epithelial cells [3,22,23].

Tobacco consists of nitrosamines, like N-nitrosonor nicotine, which are known to cause malignancies [24]. In many systems, these materials induced activation in multiple organs, it may result in chromosomal anomalies, sister chromatid exchange, gene mutation, oxidative DNA adduct production, DNA strand breaking, and the formation of micronuclei. [25]. The apoptosis mechanism results in cell death through multiple stages. Initially, it involves the destruction of cell junctions and membrane structures. Subsequently, the cytoplasm contracts, and the nucleus aggregates into small masses, which eventually disintegrate into fragments and are completely lost. These alterations can be observed in the MN assay as pyknotic, karyorrhetic, and karyolytic cells [26]. Additionally, smoke-induced apoptosis of epithelial cells contributes to the pathophysiology of conditions such as chronic obstructive pulmonary disease, peptic ulcers, and age-related macular degeneration. The most reasonable explanation for these findings is that smoking induces cellular damage by way of oxidative stress [27,28].

There is no significant link between micronucleus expression and dental caries (DMFT), except for a weak significant correlation between PK and DMFT. These findings coincide with findings which identified that the micronucleus assay and the oral hygiene indices do not significantly correlate. But the genotoxic consequence of smoking leads to higher micronuclei expression and lead to deteriorating oral health [29].

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Table 1. Micronucleus assay by Giemsa stain among smokers and nonsmokers).

Micronucleus Assay	Groups		t-test	P - Value
	Smoker Mean ± SD	Non-smoker Mean ± SD		
Micronuclei	1.91 ± 1.2	0.94 ± 1.0	3.687	0.001
Binucleated cells (BN)	0.45 ± 0.78	0.2 ± 0.4	1.73	0.09
Pyknotic cells (PK)	20.65 ± 7.5	13.65 ± 4.2	4.841	0.001
Karyorrhectic cells (KR)	0.22 ± 0.54	0.17 ± 0.45	0.476	0.636
Karyolysis cells (KL)	0.48 ± 0.65	0.14 ± 0.35	2.711	0.009
Nuclear Buds (NB)	0.17 ± 0.38	0.08 ± 0.28	1.065	0.291
Cells with Condensed Chromatin (CC)	0	0	-	-

Table 2. Correlations between DMFT and micronucleus among smokers.

Variables / Micronucleus Assay		DMFT
MN	r	-.182
	P-Value	.296
BN	r	.166
	P-Value	.341
PK	r	.099
	P-Value	.572
KR	r	-.172
	P-Value	.323
KL	r	-.148
	P-Value	.397
NB	r	-.180
	P-Value	.301

Table 3. Correlations between DMFT and micronucleus assay among non-smokers.

Variables / Micronucleus Assay		DMFT
MN	r	-.020
	P-Value	.908
BN	r	.226
	P-Value	.191
PK	r	-.399
	P-Value	.018
KR	r	.135
	P-Value	.440
KL	r	-.322
	P-Value	.059
NB	r	-.183
	P-Value	.292